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Biological Implications of Metabolic Scaling in Tobacco Hornworm (Manduca sexta)

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Biological Implications of Metabolic Scaling in Tobacco Hornworm *(Manduca sexta) Larvae*

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Abstract

Body size is closely tied to several morphological characteristics, including metabolic rate. Metabolic rate and body size are scaled in such a way that an individual's metabolic rate is equal to body weight raised to the metabolic scaling exponent b (MR = BW^b). We used open-system respirometry to measure metabolic rate in individual larvae over the course of the larval lifecycle. Larvae reared on artificial wheat germ diet had a mean scaling exponent of 0.78 ± 0.03, while larvae reared on tobacco leaves had a mean scaling exponent of 0.74 ± 0.02 (SEM). There was no significant difference between the mean scaling exponent values in the wheat germ or tobacco-fed individuals (Two sample t-test, t = 1.25, p = 0.219). The values of the scaling exponents and the inter-individual variation we observed were consistent with the results of previous studies here at Kenyon. We also examined the relationships between fecundity (egg count or testes weight) and the value of the scaling exponent and between fecundity and body weight. In general, fecundity was not significantly related to either the value of the scaling exponent or to body weight. Only female fecundity and body weight were weakly correlated.

Introduction

• Body size affects many physiological traits, including metabolism, the sum total of reactions responsible for chemical breakdown and synthesis. As an organism increases in size, metabolic rate also increases. Thus, we say that metabolic rate scales with body size.
• The metabolic scaling relationship is defined mathematically as:

Metabolic rate = $a(\text{Body Mass})^b$.

• Two competing hypotheses exist regarding a possible universal value of *b*.
• Metabolic scaling may be isometric (geometrically proportional over all developmental stages) with a scaling exponent of 0.67, as suggested by Max Rubner in 1883 (White & Seymour, 2005)
• Metabolic scaling may also be allometric (non-isometric) with a scaling exponent of 0.75, as suggested by Max Kleiber in 1932.
• Most experimental evidence, including previous work done at Kenyon, suggests that metabolic scaling is allometric; however, the value of the scaling exponent can vary from 0.4 to 1.4 (Glazier, 2005).
• *Manduca sexta* larvae are excellent model organisms for studying the metabolic scaling relationship. Larval body mass increases 10,000 fold over the course of about 2.5-3 weeks without any major changes in morphology or diet (Goodman *et al*., 1985, cited by Greenlee & Harrison, 2005).
• We addressed several questions in this study, including 1) the value of the scaling exponent *b*, 2) the scope of inter-individual variation in the value of the scaling exponent, 3) the effect of diet on the scaling relationship, 4) the effect of body weight and the scaling exponent value on nitrogen assimilation, and 5) the effect of body weight and the scaling exponent value on reproductive success, specifically fecundity.

Materials & Methods

• *Manduca sexta:* All larvae (from Carolina Biological Supply) were reared in individual plastic containers in an incubator set at 27°C with a 16L:8D photoperiod. Individuals were weighed daily and fed either artificial wheat germ diet or tobacco. All larvae were allowed to pupate and develop into adult moths. Two days after eclosion, the moths were weighed, and their abdomens were preserved in 95% ethanol. All preserved abdomens were dissected in order to evaluate fecundity. Female fecundity was evaluated in terms of mature egg count, while male fecundity was evaluated in terms of testes weight.

• *Respirometry:* Metabolic rates were measured using an open-system respirometry unit from Qubit Systems in an environmental chamber kept at 27°C. The unit was calibrated with room air (0 ppm) and a known standard sample (500 ppm). Each trial consisted of a 2-minute baseline CO_2 measurement followed by a 25-minute measurement with the larva inside an enclosed animal chamber. A respiratory quotient from Alleyne (1997) was used to convert CO_2 given off to O_2 consumed.

• *Data Analysis*: All analyses were performed with Minitab 15. The data sets were adjusted to remove outliers (based on a probability distribution plot) before the regression analyses and other tests were performed. If a data point was flagged as an outlier in the probability distribution plot, then that individual's measurements were removed from the data set entirely, and any data from the dissections were omitted as well. Comparison of mean scaling exponent values with and without the outliers indicated that the adjustments did not affect each data set's overall integrity (Two sample t-tests, t = 1.07 & p = 0.288 for the wheat germ-fed set and t = 0.55 & p = 0.590 for the tobacco-fed set).

Results

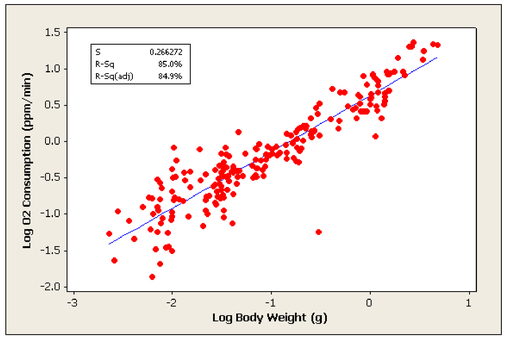

Figure 1. Metabolic scaling relationship in wheat germ-fed larvae (213 meas. of 33 indivs.). (Equation: log O_2 consumption = 0.6351 + 0.7763 log body weight, R^2 = 0.85, ANOVA p = 0.000) .

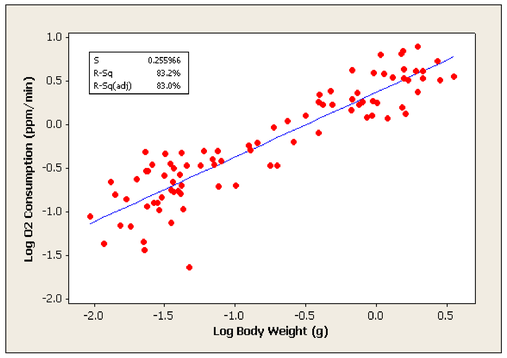

Figure 2. Metabolic scaling relationship in tobacco-fed larvae (88 meas. of 12 indivs.). (Equation: log O_2 consumption = 0.3716 + 0.7409 log body weight, R^2 = 0.83, ANOVA p = 0.000) .

Wheat germ-fed larvae had a mean metabolic scaling exponent of 0.78 ± 0.03, while tobacco-fed larvae had a mean metabolic scaling exponent of 0.74 ± 0.02 (error = SEM). The mean scaling exponent values of the two larval diet groups were not significantly different (Two sample t-test, t = 1.25, df = 39, p = 0.219).

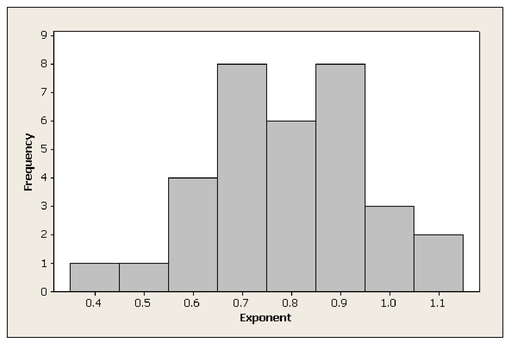

Figure 3. Distribution of metabolic scaling exponents in wheat germ-fed larvae (n = 33).

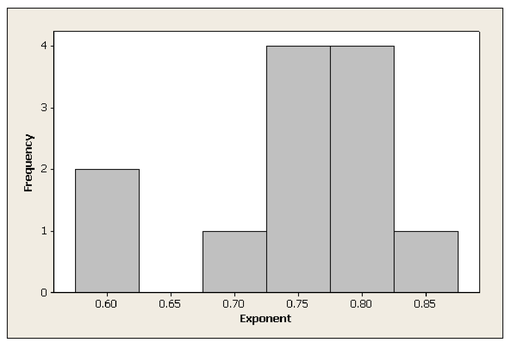

Figure 4. Distribution of metabolic scaling exponents in tobacco-fed larvae (n = 12).

The inter-individual variation in the metabolic scaling exponent values of the wheat germ-fed and tobacco-fed larvae was close to normal.

Results cont.

Relationship (Y var. & X var.)	Number of Moths	R^2 Value	P Value
Mature Egg Count & Body Weight	6	0.467	0.134
Mature Egg Count & Scaling Exponent	6	0.080	0.586
Testes Weight & Body Weight	3	0.067	0.833
Testes Weight & Scaling Exponent	3	0.025	0.841

Table 1. Fecundity and scaling in adult female and male moths. Data are based on regression analyses.

There was a weak relationship between female fecundity and body weight, but in general, fecundity was not related to either body weight or the value of the metabolic scaling exponent.

Discussion

• The mean metabolic scaling exponents for the wheat germ-fed and tobacco-fed larvae were consistent with Kleiber's proposed value of *b*, suggesting that scaling in *M. sexta* is allometric. Our *b* values are actually slightly lower than the average value previously observed here at Kenyon (*b* = 0.90) (Vreede, Stearns, & Melbarde, unpublished work).
• The similarity between the metabolic scaling exponents of the wheat germ-fed and tobacco-fed larvae suggests that metabolic scaling may not be tied to diet type. This makes sense in light of the fact that several birds and mammals with different diets have metabolic scaling exponents with similar values of about 0.75 (West & Brown, 2005).
• The inter-individual variation in the scaling exponent values was consistent with the natural range of variation described by Glazier (2005).
• The results of the female fecundity study were not consistent with previous work at Kenyon that examined the relationship between mature egg count and the scaling exponent (Vreede, Barrett, & Deimeke, unpublished work). The extremely small sample sizes (mostly due to the high mortality occurring during the pupal stage) are probably responsible for the inconsistencies observed.
• The individuals omitted from the data sets were not representative of the population. Most individuals who were flagged as containing outlier data either died early or were generally unhealthy and were not growing at the same rate as the healthy larvae.
• Larval health has been and continues to be an issue in our studies. Several larvae became ill or died due to a mold infection. This detrimental effect on health may have led to some of the outlier measurements noted above.
• Future studies should attempt to correct the issues mentioned previously, including using more care with the prevention and response to mold problems and increasing the sample sizes used in the fecundity studies (by reducing mortality during pupation).

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Manduca pictures from www.rhosgobel.blogspot.com

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